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PHYSIOLOGICAL IMPACT OF DREDGED SEDIMENT ON TWO BENTHIC SPECIES

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16. ABSTRACT

Several international commissions have recently called for effective short-term biological response measurements which can adequately detect the effects of environmental concentrations of contaminants. Such methods must be relevant to ecological fitness and must have a predictive capability.

This report describes efforts to develop a direct comparison of the effects of Black Rock Harbor dredged material on the bioenergetics of juvenile Nephtys incisa exposed in the laboratory and of individuals obtained in the field from a controlled disposal of Black Rock Harbor material. Exposure regimes used in the laboratory studies were similar to the exposure environments that have been predicted around the BRH disposal site in Long Island Sound. In addition the effects of exposure to suspended BRH material on the bioenergetics of the mysid, Mysiodopsis bahia were also determined.

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SECTION 1

INTRODUCTION

The disposal of dredged material can pose problems to the well being and survival of benthic communities on or near the site of disposal. Problems range from the near complete but temporary loss of the infaunal community at the impact zone to the potential long-term impact caused by the bioaccumulation of contaminants from the sediments. Further problems arise from the possible effect on human health through biomagnification of toxic substances through the food chain to edible commercial species.

The management of material through ocean disposal becomes difficult at best when one attempts to assess any eventual consequences to the environment caused by the disposal of a particular type of material. Predictive capabilities, based on accepted scientific evidence are the cornerstone to a sound ocean disposal management plan. One such management plan incorporates a hazard assessment scheme in which a step-sequence assessment is made of potential hazards associated with a particular material (Duthie, 1977; Lloyd, 1980).

A management plan of this type is currently being analyzed in a joint United States Environmental Protection Agency and Army Corps of Engineers research program in which the effects of dredged material from Black Rock harbor (BRH), Connecticut on a Long Island Sound benthic species will be assessed in the laboratory prior to disposal operations (Gentile et al., 1985). Following disposal, a field study will be conducted to verify the response observed in the laboratory and determine the accuracy of the laboratory prediction.

This research program offers a unique opportunity to sequentially develop and determine the predictive capacity of a variety of biological effects tests.

Effective short-term biological response measurements which can adequately detect the effects of environmental concentrations of contaminants recently have been called for by several international commissions (ICES, 1978; McIntyre and Pearce, 1980). In order to be of value the measurements must have some relevance to ecological fitness. In addition, these relatively short-term laboratory efffects tests (usually < month) must have a predictive capility which allows for the estimation of the degree of ecological change which will take place.

An effects measurement technique which may satisfy the preceding criteria is the determination of biological energy balances (Capuzzo and Lancaster, 1981; Johns and Pechenik, 1980; Johns and Miller, 1982; McKinney, 1982) along with its corollaries, including scope for growth (Warren and Davis, 1967; Bayne, 1975). Previous studies using these principles have found a reasonable correlation between changes in energy balances or scope for growth and changes in population fitness (Bayne et al., 1979; Gilfillan, 1980). In a series of detailed field studies, for example, Gilfillan et al. (1976) found a reduced scope for growth in Mya arenaria collected from oil-impacted sites when compared to individuals from nearby, relatively clean reference populations. These data were related to and predictive of eventual changes in population structure which were observed in the impacted sites. Changes in population structure that Gilfillan could relate to the reduced scope for growth included reductions in yearly growth rate and populations density.

This report describes efforts to develop a direct comparison of the effects of Black Rock harbor material on the bioenergetics of juvenile Nephtys incisa exposed in the laboratory and individuals obtained in the field from a controlled disposal of Black Rock Harbor material. Exposure regimes used in the laboratory studies were similar to the exposure environments that have been predicted around the BRH disposal site in Long Island Sound (Munns, 1986).

In addition to the research conducted with Nephtys incisa, the effects of exposure to suspended BRH material on the bioenergetics on the mysid, Mysidopsis bahia were also determined. M. bahia is currently being used in the Field Verification Program as a laboratory surrogate for crustaceans that may be found in and around the BRH disposal site (Gentile et al., 1985). The research presented here for the mysid was conducted in conjunction with the population dynamics research of Gentile and his co-workers.

SECTION 2

MATERIALS AND METHODS

LABORATORY STUDIES WITH NEPTHYS INCISA

Sediment Sources and Exposue Systems

The sediment used as control sediment in these studies was collected from the South Reference (REF) site in Long Island Sound (41 7.95"N and 72 52.7"W), the same site from which the worms were collected (Fig. 1). The sediment was collected by Smith-MacIntyre grab (0.1m), press sieved through a 2mm sieve and stored at 4°C until used. Black Rock Harbor sediment (BRH) was collected with a gravity box corer (0.1m) to a depth of 1.21m, throughly mixed, and refigerated. Further detail on the handling of sediment used in these studies can be found in Rogerson et al., 1985.

The worms were exposed to suspended particles at a nominal concentration of 200mg/l using various combinations of REF and BRH material (Table 1). The particles were delivered to the exposure system using the composite dosing system (Fig. 2) described by Rogerson et al. (1985) except that a micro-processor was used to control and deliver the sediment. Prior to being placed in the composite dosing system the control and BRH sediments were oxidized for three days using compressed air to provide a closer approximation of the oxidation state of suspended sediments that would be found in Long Island Sound.

The exposure system used for Nephtys incisa consisted of the composite dosing system, a suspended sediment proportional dilutor which mixed and delivered the appropriate mixture of REF and BRH material, and the treatment containers. The treatment containers were 19-l polycarbonate

Table 1

Total suspended particle load in the dosing system used for *Nephtys incisa*

Exp.	Treatment	Nominal Concentration (mg/l)	Measured Total Load (mg/l)*
1;2	100% REF	200 REF	167 ± 58
	75% REF/25% BRH	150 REF/50 BRH	169 ± 18
	50% REF/50% BRH	100 REF/100 BRH	181 ± 20
	25% REF/75% BRH	50 REF/150 BRH	194 ± 17
	100% BRH	200 BRH	199 ± 57
3	100% REF	200 REF	203 ± 24
	50% REF/50% BRH	100 REF/100 BRH	184 ± 21
	100% BRH	200 BRH	182 ± 24
4	100% REF	200 REF	194 ± 13
	50% REF/50% BRH	100 REF/100 BRH	188 ± 18
	100% BRH	200 BRH	192 ± 25

* Mean ± 1 S.D.

bottles which had the tops removed to allow free access to the inside. Approximately 2-L of REF sediment was added to each to provide a substrate for worms to burrow. Each container was fitted with a low pressure airlift to keep the dosed sediments in suspension (Fig. 3).

In all laboratory experiments, a minimum of 20 juvenile worms of approximately the same size were placed in each treatment. The bedded sediment in all treatments was reference sediment (REF) that had been collected in Long Island Sound. After the appropriate exposure period, the worms were sieved from the mud, counted and saved for physiological measurements.

During the exposures worms were offered prawn flakes (Aquatic Diet Technology, Inc., Brooklyn, NY) as a food source. Previous research (Paul Schauer, personal communication) has indicated that laboratory holdings of N. incisa grow better when offerred prawn flakes than when left without. It is unclear, however, whether the prawn flakes are used directly as a food source or if it acts as a substrate for bacterial growth, the bacteria then being utilized in turn as the food source.

Experimental Organisms

Nephtys incisa used in all laboratory experiments were collected from Long Island Sound in the vicinity of the South Reference site. Bottom samples were collected using a Smith-MacIntyre grab. The upper 5 cm of the grabs were placed in a tub for transport back to the laboratory. In the laboratory the worms were sieved and worms which passed through a 2mm sieve but were retained on a 0.37mm sieve were kept for laboratory experimentation.

Following this, individuals were visually separated into relative size classes for experiments. Although somewhat subjective, careful visual separation leads to a coefficient of variation in dry weight of only 24%. Other more accurate sizing techniques, such as wet weight determinations employing a wet weight to dry weight regression curve are time consuming and, more importantly, may cause physical damage to the worms because of the handling required.

All experiments were conducted at 20°C. Laboratory acclimation for Nephtys incisa collected at 20 ± 3°C was a minimum of three days to allow for adjustments to laboratory holding conditions. For worms collected at temperatures below 16°C, acclimation included a 1-2°C increase in temperature per day until 20°C was attained.

Physiological Measurements

At the appropriate sampling time, 15-20 worms were taken from the experimental conditions to assess their physiological state. Individual respiration rates were determined prior to rinsing, drying and weighing. With some studies, percent protein, carbohydrate, lipid, and ash were determined from homogenous samples of ground tissue following physiological determinations.

Production

In all experiments, juvenile worms were used so that changes in dry weight reflected only changes in growth (production). At the beginning of an experiment a subsample of individuals (10-15) was taken to estimate initial worm weight. The worms were quickly rinsed in deionized water, dried at 60°C for 24h then weighed on a Perkin-Elmer Ad-2Z Autobalance

to the nearest 1 µg. Changes in growth were computed as being the difference in dry weight from the beginning to the end of an experiment. Energy values for the tissue were determined using a wet oxidation technique in the presence of an acid-dichromate mixture (Maciolek, 1962).

Respiration

For Nephtys incisa, routine rates of oxygen consumption were measured using a 3cc syringe containing a small amount of sediment (0.5 to 0.75cc) as a respirometer (Fig. 4). A worm was placed in each syringe with 1.5cc of air-equilibrated seawater. After an appropriate period of time (usually 1-1.5h), the oxygen concnetration of 1cc of the seawater was determined by injecting the sample onto the face of a Radiometer thermostated sampling cell. The syringe was then refilled to 1.5cc, and the new oxygen concentration recalculated. This procedure was repeated up to three times per worm. A control syringe respirometer containing an appropriate amount of sediment was included in each experiment. A discussion of the suitability of this method for determining the oxygen consumption rates of Nephtys incisa is presented by Johns and Gutjahr-Gobell (1984). Energy expanded during routine metabolism was considered to be the average oxygen consumption rate during the experimental period times the energy equivalent for oxygen (4.80 cal/ml; Elliot and Davison 1975).

Ammonia Excretion

Following their use in the respiration tests, individual N. incisa were placed in 6 ml of filtered seawater without sediment for approximately 3h to determine ammonia excretion rates. Ammonia concentrations were

determined according to the technique of Bowen and Holm-Hansen (1980). Calories lost per unit time as excretion rate of an individual worm during the experimental period by the heat of formation of ammonia (0.62 cal/mg; Elliot and Davison, 1975).

Proximate Analysis

For those experiments where it may apply, worms in groups of three were ground to a homogenous powder for biochemical analysis following the determination of dry weight. Separate subsamples of the powdered tissue were used to estimate (1) protein and carbohydrate content, (2) total lipids, and (3) ash content. The protein assay used in this study was a modification of the Lowry technique (Hartree, 1972), while the carbohydrate assay used was a standard colometric technique (Dubois et al., 1956) modified for micro-analysis.

Total lipids were measured using a gravimetric technique modified after Folch et al. (1957) in which lipids were extracted using a chloroform-methanol mixture. Ash content was determined by combusting a weighed sample of powdered tissue at 500°C for 24h. The samples were then reweighed.

In addition to the laboratory exposures to BRH material, a seasonal study was conducted with Nephtys incisa juveniles to develop baseline physiological and biochemical changes expected in Long Island Sound worm populations. Sediment and juvenile worms used were collected from the South Reference site. A total of 8 sampling cruises were made between October 1982 and September 1983 (Table 2). Bottom samples were collected using a Smith-MacIntyre grab. At the time of sampling, sea water temperature and salinity measurements were made one meter above the bottom. The temperature of the upper 5cm of sediment in the grab was also taken.

Table 2

Collection times and bottom sediment temperature for samples collected between October 1982 and September 1983

Collection	Date	Bottom Temperature (°C)
1	October 1982	20.0
2	November 1982	11.0
3	January 1983	5.2
4	March 1983	5.4
5	April 1983	7.6
6	June 1983	12.0
7	July 1983	21.0
8	September 1983	20.0

In the laboratory a minimum of 40 worms were placed in 4-L of press-sieved reference sediment for 28 days. During this period, the water temperature and salinity of the experimental boxes was monitored daily. The worms were offered prawn flake as a food source.

At the beginning of an experiment, a random sample of 15 worms were selected to provide an initial measure of maintenance costs, biochemical composition, and tissue weight. At the end of 28 days, a minimum of 20 worms were used to determine a final measure of maintenance costs, biochemical composition, and tissue production.

FIELD STUDIES WITH NEPHTYS INCISA

Field Collection

Collections of Nephtys incisa juveniles were made once prior to the initial disposal of BRH dredged material and at prescribed sampling times (Table 3). These samples constituted the field exposure time course that was approximately replicated in the laboratory. Four stations were occupied during sampling and included the South Reference site, 200, 400, and 1000 meters east of the center of the disposal mound (Fig. 5). Stations South reference, 200ME, and 400ME were approximated in the laboratory by the exposure regimes of 200mg/l control sediment, 200mg/l of a 50 percent mix of REF and BRH sediment, and 200mg/l of BRH sediment, respectively.

Worms were collected using a Smith-MacIntyre grab. Whole unsieved grabs were transported to the laboratory for sieving. The sediment was sieved using ambient sea water and juveniles of the appropriate size were collected. Respiration rates of individual worms were determined

Table 3

Field collections of *Nephtys incisa* at the BRH disposal site

Collection Period	DATE (Day/Month/Yr)	Bottom Water Temperature (°C)	Stations Sampled
Pre-Disposal	12-04-83	4.5	400E,1000E,SREF
T+2	06-06-83	10.5	200E,400E,1000E,SREF
T+8	19-07-83	18.4	200E,400E,1000E,SREF
T+12	02-08-83	19.6	200E,400E,1000E,SREF
T+16	09-08-83	21.3	200E,400E,1000E,SREF
T+28	14-12-83	7.2	200E,400E,1000E,SREF
T+40	15-03-84	0.8	200E,400E,1000E,SREF
T+55	21-06-84	11.4	200E,400E,1000E,SREF
T+74	10-10-84	17.1	200E,400E,1000E,SREF
T+117	26-06-85	--	200E,400E,1000E,SREF

using the syringe respirometers containing a small amount of surficial sediment from the appropriate collection station. Following the determination of respiration rates, individual worms were placed in filtered seawater to determine ammonia excretion rates. Finally, the worms were rinsed and dried to determine dry weight. All physiological measurements were made at ambient conditions. Techniques and analytical procedures used in this portion of the study were similar to those used in the laboratory phase of this study.

LABORATORY STUDIES WITH MYSIDOPSIS BAHIA

Exposure System

The exposure system consisted of the composite dosing system described earlier, a suspended sediment proportional dilutor which mixed and delivered small quantities of REF/BRH sediment to treatment containers and individual exposure chambers (Gentile et al., 1985). The delivery system was designed to maintain the same concentration of suspended particles with different ratios of REF and BRH sediment being delivered to each treatment container. In all treatments, the total suspended particle level was maintained at a nominal concentration of 300 mg/l.

Exposure chambers consisted of wide-mouth glass jars, 8.5 cm high X 7.5 cm wide with two 3.2 cm holes drilled in opposite sides (Fig. 6). The holes were screened with 250 mm Nitex screen netting. A total of 4 exposure chambers could be maintained in each treatment container which was consisted of a 190 x 100mm crystilizing dish fitted with automatic umbrella siphons. The siphons provide for turnover of suspended particles within the treatment containers and particle exchange between

the treatment container and the four exposure chambers. The exposure chambers were suspended within the crystilizing dish by means of a glass tab glued to the top rim of the exposure chamber (Fig. 6). The configuration of the complete exposure system allowed for replicate treatment containers for each level of suspended particles tested (total of 8 exposure chambers per treatment). Detailed information on the design and functioning of the mysid exposure system can be found in Gentile et al. (1985).

Culture

Mysidopsis bahia is an epibenthic crustacean that lends itself to laboratory culture (Nimmo and Hamaken, 1982). The life cycle of this mysid is relatively short (25 days at 25°C) and can be easily maintained in the laboratory. Stock populations are maintained in 76-l glass aquaria continuously supplied with filtered (15 μ) seawater at a temperature of 25 $\pm$ 2°C and a salinity of 28 $\pm$ 2° ppt. The cultures were fed ad libitum with 24h post hatch Artemia salina nauplii. To obtain newly-hatched juveniles for experimentation, gravid females are segregated from the rest of the stock population, held overnight, and newly released juveniles harvested.

Experimental Design

Two experiments of 15 days length were conducted between December 1983 and February 1984. In both experiments, 15 newly-released juvenile mysids were placed in each of 8 exposure chambers per treatment. On selected sampling days a given number of individuals were harvested from each treatment (randomly selected from the 8 exposure chambers) to estimate

physiological performance and determine growth rate. The sampling times and number of individuals harvested on each occasion is presented in Table 4.

Table 4

Sampling times and the number of mysids harvested during the 15-day experimental period

Day	Treatment	Number of mysids harvested
3	100% REF	42
	88% REF/12% BRH	39
	50% REF/50% BRH	42
	100% BRH	42
5	100% REF	39
	88% REF/12% BRH	42
	50% REF/50% BRH	42
	100% BRH	30
6	100% REF	42
	88% REF/12% BRH	42
	50% REF/50% BRH	42
	100% BRH	42
9	100% REF	28
	88% REF/12% BRH	28
	50% REF/50% BRH	28
15	100% REF	14
	88% REF/12% BRH	14
	50% REF/50% BRH	14

Physiological performance was based on an evaluation of the rates of feeding, respiration, ammonia excretion, and growth of mysids from

each treatment. Significant differences (at $p = 0.05$) in the various rates from those for mysids maintained in tne REF suspended particle treatment were considered as an indication of changes in physiological performance.

Physiological Measurements

Feeding rate was determined by placing an individual mysid into 5ml of filtered seawater to which a given number of newly-hatched Artemia nauplii had been placed. Following a 24h period, the difference between the number of living Artemia remaining and the initial number placed in the beaker was used an estimate of the feeding rate. A control which consisted of a beaker with only the prescribed amount of Artemia was included in each series of feeding determinations.

Respiration rates and excretion rates were determined on groups of mysids; the number of mysids in each group dependent upon the size of the individuals. The syringe respirometer described earlier for the N. incisa studies was also used with the mysids, except that a larger syringe (10cc) was employed and no sediment was added to the syringe. In addition to determining oxygen consumption rates using the syringe respirometer, ammonia excretion rates were also determined on a 5cc sample of water taken from the syringe after determination of the respiration rate rather than in a separate volume of water as it was in the polychaete studies.

Following the determination of respiration, ammonia excretion, and feeding rates, the mysids were individually rinsed in deionized water, placed on pre-weighed pans, dried at 60°C for 24h, and then re-weighed. Tissue growth was taken as the difference between the weight of each mysid and the mean dry weight for the mysids sampled in the previous period.

SECTION 3

RESULTS

LABORATORY STUDIES WITH NEPHTYS INCISA

A total of four laboratory experiments were conducted with Nephtys incisa juveniles. In the first two experiments (Exp. 1 and Exp. 2), worms were exposed to oxidized REF and BRH suspended particles in various combinations at a concentration of 200 mg/l for a period of 10 days. In the other two experiments (Exp. 3 and Exp. 4), juvenile worms were exposed to identical suspended particle conditions but the length of the experiment was 28 days for Exp. 3 and 42 days for Exp 4.

In Exps. 1 and 2 the only time worms were sampled for physiological measurements was at the end of the 10 day exposure. With Exp. 3, however, an intermediate sample was taken at day 14, while with Exp. 4 an intermediate sample was taken at day 28. The intermediate samples provided comparative data to at least one of the sampling times of the previous experiment. For example, data from the day 28 intermediate sample of Exp. 4 could be compared with the 28 day results of Exp. 3.

Production

In Exp. 1 exposure to concentrations up to 200 mg/l of BRH suspended particles did not have a significant effect on the growth of Nephtys incisa juveniles (Table 5). In a repeat of this experiment (Exp. 2), however, significant differences in growth were found between the treatments. Worms maintained in both the 100-percent BRH and 25-percent REF/75-percent BRH treatments showed significant reductions in growth

Table 5

Changes in dry weight of Nephtys incisa juveniles exposed to various suspended particle conditions for 10 days

Exp	Treatment	Dry Weight* Initial mg	Final mg	Change In Weight Gp** (%)
1	100% REF	2.030 ± 0.434	2.151 ± 0.273	A +5
	75% REF/25% BRH	2.030 ± 0.434	1.955 ± 0.325	A -4
	50% REF/50% BRH	2.030 ± 0.434	1.741 ± 0.299	A -28
	25% REF/75% BRH	2.030 ± 0.434	1.756 ± 0.486	A -14
	100% BRH	2.030 ± 0.434	1.783 ± 0.198	A -12
2	100% REF	1.263 ± 0.159	1.372 ± 0.217	A +9
	75% REF/25% BRH	1.263 ± 0.159	1.276 ± 0.205	A +1
	50% REF/50% BRH	1.263 ± 0.159	1.228 ± 0.279	A,B -3
	25% REF/75% BRH	1.263 ± 0.159	1.207 ± 0.106	B -5
	100% BRH	1.263 ± 0.159	1.072 ± 0.126	B -15

* Means ± 1 S.D.

** Gp=Grouping letter. Means having the same Gp are not significantly different

compared to worms maintained in the other treatments (Table 5). Juveniles from the 100-percent REF treatment increased their weight by an average of 9 percent during the 10 days while in the 100-percent BRH condition worms lost an average of 15 percent of their initial weight.

Increasing the length of exposure had a significant effect on the growth response of Nephtys incisa juveniles (Tables 6 and 7). Although a 14 day exposure (Exp. 3) did not result in a significant difference between the treatments, significant differences were evident by day 28 (Table 6). Worms from the 100-percent REF treatment gained an average dry weight of 6 percent while those worms from the 50-percent REF/50-percent BRH and 100-percent BRH treatments lost an average of 17 and 23 percent, respectively.

In Exp. 4 a similar level of tissue production was found at 28 days (Table 7) compared to the same time period for Exp. 3 (Table 6). Worms maintained in the 100-percent REF treatment increased in dry weight an average of 5 percent compared to 6 percent in Exp. 3, while worms exposed to the 100-percent BRH treatment lost an average dry weight of 13 percent compared to a loss of 23 percent in Exp. 3.

By day 42 all three treatments were significantly different from one another (Table 7). Worms mainatined in the 100-percent REF condition increased an average of 20 percent in dry weight, while individuals from the 50-percent REF/50-percent BRH treatment and 100-percent BRH treatments lost 16 and 28 percent, respectively.

Table 6

Changes in dry weight of Nephtys incisa juveniles exposed to various suspended particle conditions for 28 days

Time	Treatment	Dry Weight* Initial mg	Final mg	Change In Weight Gp**	%
Day 14	100% REF	1.139 ± 0.172	1.088 ± 0.324	A	-4
	50% REF/50% BRH	1.139 ± 0.172	1.053 ± 0.333	A	-8
	100% BRH	1.139 ± 0.172	1.073 ± 0.264	A	-6
Day 28	100% REF	1.139 ± 0.172	1.194 ± 0.123	A	+6
	50% REF/50% BRH	1.139 ± 0.172	0.941 ± 0.156	A,B	-17
	100% BRH	1.139 ± 0.172	0.881 ± 0.168	B	-23

* Mean ± 1 S.D.

** Gp=Grouping letter. Means having the same Gp are not significantly different

Table 7

Changes in dry weight of Nephtys incisa juveniles exposed to various suspended particle conditions for 42 days

Time	Treatment	Dry Weight* Initial mg	Dry Weight* Final mg	Change In Weight Gp**	Change In Weight (%)
Day 28	100% REF	1.496 ± 0.271	1.571 ± 0.277	A	+5
	50% REF/50% BRH	1.496 ± 0.271	1.511 ± 0.071	A	+1
	100% BRH	1.496 ± 0.271	1.273 ± 0.110	B	-15
Day 42	100% REF	1.496 ± 0.271	1.800 ± 0.150	A	+20
	50% REF/50% BRH	1.496 ± 0.271	1.263 ± 0.167	B	-16
	100% BRH	1.496 ± 0.271	1.081 ± 0.243	C	-28

* Means ± 1 S.D.

** Gp = Grouping letter. Means having the same Gp are not significantly different

Respiration Rate

In Exp. 1, weight specific respiration rates of those juvenile worms exposed to 100 mg/l BRH suspended particles (50-percent BRH treatment) or more for 10 days were significantly higher than those rates for worms exposed to particles containing less than 50 mg/l BRH (25-percent BRH treatment; Table 8).

Table 8

Weight specific respiration rate of Nephtys incisa juveniles exposed to various suspended particle conditions for 10 days

Exp.	Treatment	Respiration rate* ul02/mg/hr	N**	Gp***
1	100% REF	1.04 ± 0.25	20	A
	75% REF/25% BRH	1.09 ± 0.26	16	A
	50% REF/50% BRH	1.52 ± 0.33	16	B
	25% REF/75% BRH	1.53 ± 0.43	18	B
	100% BRH	1.42 ± 0.25	20	B
2	100% REF	1.12 ± 0.36	18	A
	75% REF/25% BRH	1.48 ± 0.41	20	B
	50% REF/50% BRH	1.55 ± 0.30	20	B
	25% REF/75% BRH	1.70 ± 0.27	20	B
	100% BRH	1.65 ± 0.24	20	B

* Means ± 1 S.D.

** N = Number of determinations

*** Gp = Grouping letter. Means having the same Gp are not significantly different.

Similar results were also found in Exp. 2 except that all treatments containing BRH material exhibited weight-specific respiration rates that were significantly higher than those rate in worms from the treatment containing only REF material as suspended particles (Table 8).

In Exps. 3 and 4, significant differences in weight specific respiration rates were found between the 100-percent REF treatment and all treatments containing BRH material in the suspended phase (Tables 9 and 10).

Table 9

Weight specific respiration rate of Nephtys incisa juveniles exposed to various suspended particle conditions for 28 days

Experiment 3

Time	Treatment	Respiration rate* ul02/mg/hr	N**	Gp**
Day 14	100% REF	1.26 ± 0.34	18	A
	50% REF/50% BRH	1.57 ± 0.41	20	B
	100% BRH	1.57 ± 0.38	16	B
Day 28	100% REF	0.87 ± 0.13	16	A
	50% REF/50% BRH	1.45 ± 0.25	14	B
	100% BRH	1.17 ± 0.28	16	B

* Mean ± 1 S.D.

** N = Number of determinations

*** Gp = Grouping letter. Means having the same Gp are not significantly differrent.

This pattern was observed at both sampling times (14 and 28 days) in Exp. 3 (Table 9) and at the 28 day sample in Exp. 4 (Table 10). The only exception to this pattern was after 42 days of exposure. In this case, no significant difference was found between the 100-percent REF treatment and the 50-percent REF/50-percent BRH treatment. The respiration rate of worms from both of these treatments, however, were significantly different from those at the 100% BRH treatment.

Table 10

Weight specific respiration rate of Nephtys incisa juveniles exposed to various suspended particle conditions for 42 days

Experiment 4

Time	Treatment	Respiration rate* ul02/mg/hr	N**	Gp***
Day 28	100% REF	1.03 ± 0.15	16	A
	50% REF/50% BRH	1.27 ± 0.17	14	B
	100% BRH	1.31 ± 0.14	8	B
Day 42	100% REF	0.92 ± 0.25	16	A
	50% REF/50% BRH	0.98 ± 0.31	16	A
	100% BRH	1.28 ± 0.19	16	A

* Means ± 1 S.D.

** N = Number of determinations

*** Gp = Grouping letter. Means having the same Gp are not significantly different.

Ammonia Excretion Rate

No significant differences were found in the weight specific ammonia excretion rate between the various treatments in Exp. 1 (Table 11). In Exp. 2, however, significantly lower weight specific excretion rates were found at 10 days for those juveniles in treatments where the BRH level was greater than 50 percent of the total suspended particle load (Table 11).

Table 11

Weight specific ammonia excretion rate of Nephtys incisa juveniles exposed to various suspended particle conditions for 10 days

Exp	Treatment	Excretion rate* ugNH4N/mg/hr	N**	Gp**
1	100% REF	.009 ± .002	20	A
	75% REF/25% BRH	.008 ± .002	16	A
	50% REF/50% BRH	.008 ± .002	16	A
	25% REF/75% BRH	.008 ± .004	18	A
	100% BRH	.009 ± .002	20	A
2	100% REF	.008 ± .003	18	A
	75% REF/25% BRH	.008 ± .003	20	A
	50% REF/50% BRH	.004 ± .001	20	B
	25% REF/75% BRH	.004 ± .001	20	B
	100% BRH	.004 ± .001	20	B

* Means ± 1 S.D.

** N = Number of determinations

*** Gp = Grouping letter. Means having the same Gp are not significantly different

In Exp. 3 no significant differences in excretion rate were found between the treatments at day 14, however, by day 28 worms from the 100-percent BRH treatment exhibited significantly lower excretion rates compared to the 100-percent REF and 50-percent REF/50-percent BRH groups (Table 12).

Table 12

Weight specific ammonia excretion rate of Nephtys incisa juveniles exposed to various suspended particle conditions for 28 days

Experiment 3

Time	Treatment	Excretion rate* ugNH4N/mg/hr	N**	Gp***
Day 14	100% REF	.011 ± .002	18	A
	50% REF/50% BRH	.009 ± .002	20	A
	100% BRH	.010 ± .004	16	A
Day 28	100% REF	.013 ± .004	16	A
	50% REF/50% BRH	.011 ± .002	14	A
	100% BRH	.008 ± .002	16	B

* Means ± 1 S.D.

** N = Number of determinations

*** Gp = Grouping letter. Means having the same Gp are not significantly different

In Exp. 4 significant differences in ammonia excretion rate was found between the treatments at both the 28 and 42 day sampling times. At 28 days worms from the 100-percent BRH treatment exhibited significantly lower ammonia excretion rates than did worms from the other two treatments. At 42 days a similar pattern was found except that the worms from the 50-percent REF/50-percent BRH treatment were statistically intermediate to the 100-percent REF and 100-percent BRH treatments (Table 13).

Table 13

Weight specific ammonia excretion rate of Nephtys incisa juveniles exposed to various suspended particle conditions for 42 days

Experiment 4

Time	Treatment	Excretion rate* ug NH4N/mg/hr	N**	Gp***
Day 28	100% REF	.009 ± .004	16	A
	50% REF/50% BRH	.011 ± .003	14	A
	100% BRH	.006 ± .002	8	B
Day 42	100% REF	.012 ± .002	16	A
	50% REF/50% BRH	.009 ± .003	16	A,B
	100% BRH	.007 ± .003	16	B

* Means ± 1 S.D.

** N = Number of determinations

*** Gp = Grouping letter. Means having the same Gp are not significantly different.

Partitioning of Energy

The preceeding physiological data were used to calculate the efficiency at which available energy was partitioned between growth (production [P]) and maintenance costs (respiratory energy [R]). In the two experiments of ten days length (Exp. 1 and 2), no significant differences were found in maintenance costs between the treatments (Table 14). Significant differences, however, were found in the amount of energy partitioned to growth during the 10 day exposure period. In both experiments, worms from the 100-percent REF treatment converted a significant amount of available energy to new tissue while worms from the 100-percent BRH treatment lost weight during the experiment (Table 14).

Table 14

Cummulative energy values for production and maintenance costs of Nephtys incisa juveniles exposed to various suspended particle conditions for 10 days

Exp	Treatment	Production* J**	Gp***	Maintenance Costs* J**	Gp***
1	100% REF	+1.45 ± 0.17	A	7.12 ± 2.15	A
	75% REF/25% BRH	-0.90 ± 0.15	B	6.74 ± 1.54	A
	50% REF/50% BRH	-3.41 ± 0.31	C	8.34 ± 1.70	A
	25% REF/75% BRH	-3.29 ± 0.92	C	8.29 ± 2.24	A
	100% BRH	-2.96 ± 0.33	C	8.10 ± 1.48	A
2	100% REF	+1.31 ± 0.21	A	4.99 ± 1.03	A
	75% REF/25% BRH	+0.16 ± 0.03	B	6.11 ± 1.83	A
	50% REF/50% BRH	-0.42 ± 0.09	C	6.11 ± 1.71	A
	25% REF/75% BRH	-0.67 ± 0.06	C	6.54 ± 0.97	A
	100% BRH	-2.29 ± 0.27	D	5.67 ± 0.99	A

* Means ± 1 S.D.

** J = Joules

*** Gp = Grouping letter. Means having the same Gp are not significantly different.

A similar pattern in energy partitioning was found in 28 day and 42 day experiments (Table 15 and 16). In both of these experiments, significant differences in the amount of energy converted to new tissue can be seen among the treatment groups with the 100-percent REF group converting a greater amount of energy to tissue than the worms from the other treatments.

Table 15

Cummulative energy values for production and maintenance cost of *Nephtys incisa* juveniles exposed to various suspended particle conditions for 28 days

Time	Treatment	Growth* J**	Gp***	Respiratory Energy Expenditure* J**	Gp***
Day 14	100% REF	-0.61 ± 0.18	A	10.72 ± 0.86	A
	50% REF/50% BRH	-1.03 ± 0.33	A	10.45 ± 1.94	A
	100% BRH	-0.79 ± 0.19	A	10.25 ± 2.57	A
Day 28	100% REF	+0.66 ± 0.07	A	13.89 ± 1.11	A
	50% REF/50% BRH	-2.38 ± 0.39	B	10.83 ± 2.25	B
	100% BRH	-3.10 ± 0.59	B	10.45 ± 2.14	B

* Means ± 1 S.D.

** J = Joules

*** Gp = Grouping letter. Means having the same Gp are not significantly different

Table 16

Cummulative energy values for production and maintenance costs of *Nephtys incisa* juveniles exposed to various suspended particle conditions for 42 days

Time	Treatment	Growth* J**	Gp***	Respiratory Energy Expenditures* J**	Gp***
Day 28	100% REF	+0.90 ± 0.16	A	27.28 ± 3.52	A
	50% REF/50% BRH	+0.18 ± 0.01	B	25.38 ± 3.26	A,B
	100% BRH	-2.68 ± 0.23	C	20.90 ± 4.63	B
Day 42	100% REF	+3.65 ± 0.30	A	32.70 ± 6.82	A
	50% REF/50% BRH	-2.80 ± 0.31	B	36.04 ± 6.18	A
	100% BRH	-4.98 ± 1.12	C	25.45 ± 4.36	B

* Means ± 1 S.D.

** J = Joules

*** Gp = Grouping letter. Means having the same Gp are not significantly different.

Comparison of the net growth efficiency of *N. incisa* juveniles from the various treatments in all experiments indicated that worms maintained in the 100-percent treatment were the most efficient (Range: ± 4.2 to ± 21.8 percent) at converting available energy to new tissue (Tables 17, 18 and 19). Worms maintained in 100-percent BRH were the least efficient (Range: -8.2 to -60.7 percent) and in fact lost weight by the end of

the exposure period in all experiments. Worms maintained in combinations of REF and BRH suspended particles exhibited net growth efficiencies that were intermediate to the net growth efficiencies found in the 100 percent REF and 100-percent BRH treatments.

Table 17

Net growth efficiency of Nephtys incisa juveniles exposed to various suspended particle conditions for 10 days

Exp	Treatment	Net Growth Efficiency (%)*	Gp**
1	100% REF	+16.9 ± 4.2	A
	75% REF/25% BRH	-12.5 ± 3.7	B
	50% REF/50% BRH	-35.6 ± 11.5	C
	25% REF/75% BRH	-32.5 ± 12.7	C
	100% BRH	-28.9 ± 8.6	C
2	100% REF	+21.8 ± 6.5	A
	75% REF/25% BRH	+ 2.5 ± 0.8	B
	50% REF/50% BRH	- 7.4 ± 2.7	C
	25% REF/75% BRH	-11.4 ± 2.9	C
	100% BRH	-60.7 ± 18.2	D

* Means ± 1 S.D.

** Gp = Grouping letter. Means having the same Gp are not significantly different.

Table 18

Net growth efficiency of Nephtys incisa juveniles exposed to various suspended particle conditions for 28 days

Time	Treatment	Net Growth Efficiency (%)*	Gp**
Day 14	100% REF	-6.3 ± 2.4	A
	50% REF/50% BRH	-11.3 ± 3.3	A
	100% BRH	-8.2 ± 2.6	A
Day 28	100% REF	+4.5 ± 1.4	A
	50% REF/50% BRH	-28.2 ± 7.1	B
	100% BRH	-35.4 ± 10.6	B

* Means ± 1 S.D.

** Gp = Grouping letter. Means having the same Gp are not significantly different.

Table 19

Net growth efficiency of Nephtys incisa juvenile exposed to various suspended particle conditions for 42 days

Time	Treatment	Net Growth Efficiency (%)*	Gp**
Day 28	100% REF	+4.2 ± 1.8	A
	50% REF/50% BRH	+1.0 ± 0.3	B
	100% BRH	-14.7 ± 4.9	C
Day 42	100% REF	+12.4 ± 3.6	A
	50% REF/50% BRH	-8.4 ± 2.1	B
	100% BRH	-24.3 ± 7.3	C

* Means ± 1 S.D.

** Gp = Grouping letter. Means having the same Gp are not significantly different.

SEASONAL STUDIES

Production

The amount of new tissue produced during the eight 28 day laboratory holding periods followed a cyclical pattern (Figure 7). Lowest rates of tissue production were found between the months of November and April while the highest rates were found during the months of July and September. Transition periods from low levels of tissue production to high levels of production appeared to be the months May and July. The transition period from high to low levels of tissue production was October. This pattern of growth follows the seasonal temperature cycle (Figure 7).

Respiration Rate

Weight-specific respiration rates exhibited similar seasonal variation with rates being lower in those juvenile worms maintained in the months where water temperatures were lower than those maintained at higher temperatures (Figure 8). The highest weight specific respiration rates were found in during the months of July, September, and October, while the lowest rates were found in the months of January and November.

In most instances, there was no significant difference in the weight specific respiration rate of worms at the beginning of the 28 day holding period and at the end of this time. the only exception to this was in the October sample when the temperature dropped from 20°C at the beginning to 11°C by the end of the 28 day holding period (Figure 8).

Net Growth Efficiency

Although a seasonal pattern was observed in the amount of growth that occurred during the year as well as in maintenance costs (estimated

as respiratory expenditure), little significant difference was found in calculated net growth efficiency (Fig. 9). The only exception to this was in the month of October when the mean growth efficiency was approximately 37 percent. For the remaining portion of the year the average net growth efficiency was between 15 and 20 percent.

Biochemical Analysis

Proximate analysis (worm composition based on the percentage ash, protein, total lipid, and carbohydrate) revealed that some body constituents remain fairly constant in juvenile worms throughout the year, while other constituents exhibited a seasonal pattern (Figure 10). The percentage of ash and carbohydrate were not significantly different during the course of this study and averaged 22.7 and 5.4 percent, respectively.

Percentages of total lipids and proteins, however, did exhibit variable patterns with lipid levels generally being greater between July and September while the percentage of protein was greatest in the colder months of December through March (Fig. 10).

FIELD SAMPLING FROM BRH DISPOSAL SITE

Respiration Rate

Although limited in scope, field data collected from the various stations around the BRH disposal site for weight specific respiration and ammonia excretion rates indicated that significant changes occurred following diposal (Tables 20 and 21).

Weight specific respiration rate was not significantly different from worms at the various stations prior to the disposal of BRH material

(Table 20). Following disposal, significant changes in oxygen consumption rates ocurred between the worms from the various sampling stations. Two weeks following disposal, a significant reduction in respiration rate was seen in worms collected from the two stations closest to the center of the disposal mound (200E and 400E stations). At eight weeks post-disposal, significant differences were still evident in worms from the various stations. Worms from all stations located on an easterly tract from the disposal mound exhibited significant increases in weight specific respiration rates compared to worms from the South Reference station. Additionally, worms rrom the two stations nearest the mound (200E and 400E) had significantly higher respiration rates than did worms from the 1000E station.

During the remainder of the field sampling program, a pattern of depressed weight specific respiration rates was seen in worms from the 200E and 400E stations compared to those worms from the SREF and 1000E sites. This is particularly evident during the T+16, T+55, and T+117 sampling periods.

In general, the weight specific respiration rates of worms from all sampling stations appears to follow a seasonal pattern. During the months when water temperatures are warmer (e.g., T+2, T+8, T+16, T+55, T+117), the oxygen consumption rates are higher than in months in which water temperatures are cooler (e.g., T+28, T+40, T+74). In addition, statistically significant differences in respiration rate between worms from the disposal site sampling stations appear to occur only during the sampling times when the water temperature was warmer (e.g., T+16 vs. T+28).

Table 20

Weight specific respiration rate of Nephtys incisa juveniles collected from various stations at the BRH disposal site

Time	Station	Respiration Rate* (ulO2/mg/hr)	Gp**
Pre-disposal	SREF	0.70 ± 0.28	A
	1000E	0.75 ± 0.36	A
	400E	0.88 ± 0.31	A
	200E	---	
T+2	SREF	1.18 ± 0.39	A
	1000E	1.39 ± 0.44	A
	400E	0.92 ± 0.35	B
	200E	0.72 ± 0.29	B
T+8	SREF	1.47 ± 0.52	A
	1000E	1.51 ± 0.34	B
	400E	1.06 ± 0.32	C
	200E	0.88 ± 0.24	C
T+12	SREF	1.30 ± 0.25	A
	1000E	1.22 ± 0.28	A
	400E	0.86 ± 0.32	B
	200E	0.70 ± 0.20	B

(continued)

Table 20 (continued)

T+16	SREF	1.62 ± 0.49	A
	1000E	1.43 ± 0.46	A
	400E	1.03 ± 0.26	B
	200E	0.96 ± 0.34	B
T+28	SREF	0.48 ± 0.14	A
	1000E	0.61 ± 0.20	A
	400E	0.52 ± 0.19	A
	200E	0.58 ± 0.16	A
T+40	SREF	0.52 ± 0.18	A
	1000E	0.57 ± 0.22	A
	400E	0.63 ± 0.28	A
	200E	0.47 ± 0.31	A
T+55	SREF	1.00 ± 0.35	A
	1000E	0.87 ± 0.37	A
	400E	0.93 ± 0.41	A
	200E	0.80 ± 0.20	A
T+74	SREF	1.25 ± 0.38	A
	1000E	1.45 ± 0.46	A
	400E	0.86 ± 0.30	B
	200E	0.75 ± 0.28	B

(continued)

Table 20 (concluded)

T+117	SREF	1.36 ± 0.24	A
	1000E	1.55 ± 0.39	A
	400E	1.20 ± 0.38	A,B
	200E	0.94 ± 0.34	B

* Means ± S.D.

** Gp = Grouping letter. Means with the same Gp are not significantly different.

Ammonia Excretion Rate

As with weight specific respiration rate, statistically significant changes in ammonia excretion rate were observed in worms from several sampling stations following the disposal of BRH material (Table 21). Two weeks following disposal, significant reductions in excretion rate can be seen for worms collected from the 200E and 400E sampling stations when compared to the excretion rate of worms from the South Reference site.

During the remainder of the sampling period, weight specific ammonia excretion rates were lower in worms from the 200E and 400E sampling stations compared to worms from the 1000E and SREF sites. A seasonal cycle was found for ammonia excretion rate, with rates being greater in worms collected in these months when the water temperature was warmer.

Table 21

Weight specific ammonia excretion rate of Nephtys incisa juveniles collected from various stations at the BRH disposal site

Time	Station	Excretion Rate* (ugNH4N/mg/hr)	Gp**
Pre-disposal	SREF	0.008 ± 0.004	A
	1000E	0.007 ± 0.002	A
	400E	0.009 ± 0.004	A
	200E	---	
T+2	SREF	0.013 ± 0.004	A
	1000E	0.011 ± 0.003	A,B
	400E	0.010 ± 0.004	B,C
	200E	0.008 ± 0.002	C
T+8	SREF	0.014 ± 0.003	A
	1000E	0.012 ± 0.002	A,B
	400E	0.009 ± 0.002	B
	200E	0.010 ± 0.004	B
T+12	SREF	0.012 ± 0.005	A
	1000E	0.01 ± 0.004	A
	400E	0.009 ± 0.003	A,B
	200E	0.006 ± 0.003	B

(continued)

Table 21 (continued)

T+16	SREF	0.021 ± 0.008	A
	1000E	0.016 ± 0.006	A
	400E	0.009 ± 0.004	B
	200E	0.012 ± 0.003	B
T+28	SREF	0.006 ± 0.003	A
	1000E	0.006 ± 0.004	A
	400E	0.007 ± 0.003	A
	200E	0.007 ± 0.003	A
T+40	SREF	0.007 ± 0.002	A
	1000E	0.009 ± 0.003	A
	400E	0.006 ± 0.003	A
	200E	0.006 ± 0.004	A
T+55	SREF	0.012 ± 0.002	A
	1000E	0.009 ± 0.003	A
	400E	0.011 ± 0.004	A
	200E	0.009 ± 0.002	A
T+74	SREF	0.014 ± 0.004	A
	1000E	0.016 ± 0.004	A
	400E	0.009 ± 0.003	B
	200E	0.010 ± 0.005	A,B

(continued)

Table 21 (concluded)

T+117	SREF	0.015 ± 0.004	A
	1000E	0.016 ± 0.003	A
	400E	0.010 ± 0.003	B
	200E	0.008 ± 0.002	B

* Means ± 1 S.D.

** Gp = Grouping letter. Means having the same Gp are not significantly different.

LABORATORY EXPOSURES OF MYSIDOPSIS BAHIA TO BRH MATERIAL

Feeding Rate

Exposure level to BRH suspended material and the length of exposure had a significant effect on the Artemia ingestion rates of mysids (Table 22). Within 5 days of exposure to 300 mg/l of 100-percent BRH suspended particles, mysids exhibited a significant reduction in feeding rates. This response persisted until day 7 when all the mysids from the 100-percent BRH treatment had died.

Although no significant differences were observed in feeding rates between the remaining treatments through day 6, a significant reduction in feeding rate for mysids from the 50-percent BRH treatment compared to the 100-percent REF was indicated by day 9. The reduction in feeding rate persisted to tne termination of the experiment.

Production

Both the rate and the total amount of tissue produced significantly decreased in mysids exposed to BRH suspended particles (Table 23; Fig. 11). Through day 6, mysids from the 100-percent BRH treatment exhibited significantly lower growth rates than did mysids from those treatments containing less than 50-percent BRH material in the suspended phase (Table 23). By day 9, significant differences were found between the remaining treatments with greater reductions in growth associated with increasing amounts of suspended BRH sediment.

The overall growth rate between the 100-percent REF, 88-percent REF/12-percent BRH, and 50-percent REF/50-percent BRH treated mysids were significantly different for the experimental period. Analysis of the data fitted by linear regression models indicated that there were significant differences in the slopes from the various treatments (Fig. 11).

Table 22

Food ingestion rate of *Mysidopsis bahia* exposed to various suspended particle conditions

Day	Treatment	Feeding rate* (Artemia/day)	Gp**
5	100% REF	46 ± 16	A
	88% REF/12% BRH	51 ± 10	A
	50% REF/50% BRH	32 ± 20	A,B
	100% BRH	21 ± 11	B
6	100% REF	55 ± 17	A
	88% REF/12% BRH	46 ± 5	A
	50% REF/50% BRH	36 ± 18	A
	100% BRH	2 ± 4	B
9	100% REF	76 ± 28	A
	88% REF/12% BRH	66 ± 23	A,B
	50% REF/50% BRH	44 ± 26	B
15	100% REF	110 ± 8	A
	88% REF/12% BRH	96 ± 21	A
	50% REF/50% BRH	28 ± 28	B

* Means ± 1 S.D.

** Gp = Grouping letter. Means having the same Gp are not significantly different.

Table 23

Dry weight of Mysidopsis bahia exposed to various suspended particle conditions

Day	Treatment	Dry Weight* (mg)	N**	Gp***	Change in wt (mg)
3	100% REF	0.058 ± 0.004	14	A	.036
	88% REF/12% BRH	0.049 ± 0.006	13	A	.027
	50% REF/50% BRH	0.050 ± 0.011	14	A	.028
	100% BRH	0.045 ± 0.017	14	A	.023
5	100% REF	0.069 ± 0.013	13	A	.011
	88% REF/12% BRH	0.071 ± 0.019	14	A	.022
	50% REF/50% BRH	0.061 ± 0.010	14	A	.011
	100% BRH	0.050 ± 0.006	10	B	.005
6	100% REF	0.128 ± 0.031	14	A	.059
	88% REF/12% BRH	0.095 ± 0.018	14	A	.024
	50% REF/50% BRH	0.067 ± 0.013	14	B	.006
	100% BRH	0.063 ± 0.025	14	B	.013
9	100% REF	0.245 ± 0.053	14	A	.117
	88% REF/12% BRH	0.173 ± 0.043	14	B	.078
	50% REF/50% BRH	0.096 ± 0.043	14	C	.029

(continued)

Table 23 (concluded)

15	100% REF	0.354 ± 0.067	14	A	.109
	88% REF/12% BRH	0.237 ± 0.072	14	B	.064
	50% REF/50% BRH	0.185 ± 0.066	14	C	.089

* Means ± 1 S.D.

** N = Number of determinations

*** Gp = Grouping number. Means having the same Gp are not significantly different.

Respiration Rate

No significant differences were detected in the weight specific respiration rate of mysids maintained in the various suspended particle treatments until day 6 (Table 24). At day 6, significant increases in the respiration rate of mysids from the 50-percent REF/50-percent BRH treatment were found compared to the 100-percent REF treatment. These differences persisted to the end of the experiment. Respiration rates of mysids maintained in the 88-percent REF/12-percent BRH treatment were intermediate to the other two treatments.

Table 24

Weight specific respiration rate of Mysidopsis bahia exposed to various suspended particle conditions

Day	Treatment	Respiration rate* (ulO2/mg/hr)	N**	Gp***
3	100% REF	4.10 ± 0.78	14	A
	88% REF/12% BRH	3.90 ± 0.79	13	A
	50% REF/50% BRH	3.20 ± 1.48	14	A
	100% BRH	5.12 ± 1.91	14	A
5	100% REF	5.12 ± 0.87	13	A
	88% REF/12% BRH	5.37 ± 1.08	14	A
	50% REF/50% BRH	4.02 ± 1.64	14	A
	100% BRH	4.45 ± 2.97	10	A
6	100% REF	3.08 ± 0.47	14	A
	88% REF/12% BRH	4.50 ± 1.43	14	A,B
	50% REF/50% BRH	6.28 ± 2.10	14	B
9	100% REF	1.88 ± 0.53	14	A
	88% REF/12% BRH	2.03 ± 0.81	14	A,B
	50% REF/50% BRH	2.66 ± 0.77	14	B

(continued)

Table 24 (continued)

15	100% REF	1.75 ± 0.63	14	A
	88% REF/12% BRH	2.24 ± 0.37	14	A
	50% REF/50% BRH	2.77 ± 0.44	14	B

* Means ± 1 S.D.

** N = Number of determinations.

*** Gp = Grouping letter. Means having the same Gp are not significantly different

Ammonia Excretion Rate

Compared to the other physiological parameters monitored during this study, ammonia excretion rate exhibited little changes in response to exposure to suspended BRH material (Table 25). Significant ammonia excretion rates were found only at days 9 and 15 of the experiment for mysids maintained in the 50-percent REF/50-percent BRH treatment.

Table 25

Weight specific ammonia excretion rate of *Mysidopsis bahia* exposed to various suspended particle conditions

Day	Treatment	Excretion rate* ($ugNH_4N/mg/hr$)	N**	Gp***
5	100% REF	0.02 ± 0.02	13	A
	88% REF/12% BRH	0.02 ± 0.01	14	A
	50% REF/50% BRH	0.01 ± 0.01	14	A
	100% BRH	0.05 ± 0.01	10	B
6	100% REF	0.05 ± 0.05	14	A
	88% REF/12% BRH	0.03 ± 0.03	14	A
	50% REF/50% BRH	0.03 ± 0.01	14	A
9	100% REF	0.02 ± 0.01	14	A
	88% REF/12% BRH	0.02 ± 0.01	14	A
	50% REF/50% BRH	0.07 ± 0.02	14	B
15	100% REF	0.03 ± 0.02	14	A
	88% REF/12% BRH	0.03 ± 0.01	14	A
	50% REF/50% BRH	0.08 ± 0.03	14	B

* Means ± 1 S.D.

** N = Number of determinations.

*** Gp = Gropuing letter. Means with the same Gp are not significantly different.

SECTION 4

DISCUSSION

NEPHTYS INCISA

In a previous study, Johns et al. (1985) reported that Nephtys incisa juveniles exhibited energetic modifications when exposed to BRH material under laboratory conditions. Changes attributed to exposure to BRH sediment included increased maintenance costs, reduced tissue growth and weight loss, and lowered net growth efficiency. Laboratory data reported in this study confirm and extend these findings.

The results of the present study indicate that Nephtys incisa living in relatively contaminant-free sediment (REF sediment) are physiologically affected by exposures to suspended BRH particles. The bioenergetic effects of suspended BRH material are evidenced in some physiological processes during the first 10 days of exposure when differences in tissue production and maintenance costs were noted (Table 14). Continued exposure to BRH material does not appreciably alter the types of physiological changes observed in the worms but rather magnifies the physiological effects noted by day 10 (Tables 15 and 16).

Nephtys incisa, through normal burrowing and irrigation activities, develop an exposure environment within their burrows by actively pumping water and suspended particles into the burrow system. Water entering the burrow system comes into contact with the body and respiratory surfaces of the worm. In addition, suspended particles drawn into the burrow system would tend to settle out of the water onto sediment surfaces while any water-borne contaminants would tend to become associated with

the relatively clean REF particles in the burrow walls (Davis 1983). Burrowing and feeding activity would bring the worms into contact with the contaminated particles associated with the burrow walls.

Contaminants entering the burrow system are either in a dissolved form or associated with particulates. Because of the high suspended particle levels (200 mg/l) used in these laboratory simulations, most of the contaminants would be primarily associated with the particulate fraction. Polychlorinated bipheynals levels monitored in the dosing chambers during the experiments, for example, indicate that approximately 98 percent of the total PCB present in the water column was associated with the particulate fraction (Peter Rogerson, personal communication). Furthermore, Yevich et al. (1986) report that the only histological abnormalities found in Nephtys incisa exposed to BRH material in the laboratory experiments are thickening of the epidermis and the appearence of macrophages containing black particles. They concluded that the changes in epidermal structure were due to direct contact with BRH particles.

Black Rock Harbor sediment is a highly contaminated material containing a wide variety of potentially toxic organic and inorganic compounds (Rogerson et al. 1985). Among others, BRH contains polychlorinated biphenyls (PCB; 6.8 ppm), polyaromatic hydrocarbons (PAH; 180 ppm), the pesticide Ethylan (3 ppm), as well as copper, cadmium, and chromium at concentrations of 2385, 23 and 1430 ppm, respectively. During the 42 day experiment, tissue levels were determined on worms that were sampled at day 0, 28 and 42. Levels to which each of the above compound classes were accumulated appear to be dose related, with worms from the 100-percent

BRH treatment having higher concentrations of all compounds than worms from the other two treatments (Table 26). Worms from the 50-percent REF/50-percent BRH treatment exhibited contaminant levels intermediate to the 100-percent REF and 100-percent BRH treatments.

It is not known, however, which contaminant or combination of contaminants is responsible for the physiological dysfunctions observed in Nephtys incisa. All of the six contaminants assayed exhibit exposure-dependent accumulation rates that are consistent with, and could be explainatory of, the dose response seen for the bioenergetics data (Fig. 12).

The results of the field monitoring suggest that Nepthys incisa juveniles exhibit both seasonal and sampling station specific variation in physiological functions. Cyclical changes in respiration and ammonia excretion rate found within a station follow a pattern similar to seasonal changes in water temperature. The patterns of respiration and excretion rate observed in this study are consistent with the seasonal changes in physiology and biochemistry of Nephtys incisa juveniles collected from an area adjacent to the SREF sampling site (Fig. 7, 8, 9).

Within the pattern of seasonal changes, significant differences in respiration and ammonia excretion rates between stations occurred only during periods when the water temperature was greater than 11°C. When water tmeperatures were cooler, no significant differences in weight specific respiration and ammonia excretion rates were evident in individuals collected from the various sampling stations. Depending upon the time of year, therefore, water temperature will alter the apparent physiological effects of exposure to BRH material.

In warmer months, Nephtys incisa tend to be physiologically more active, exhibiting their highest rates of growth (Fig. 7) and their greatest burrowing activity (Davis and Miller, 1979). During this time, maximum exposure to contaminated sediments might be expected as both tube irrigation frequency (exposure to suspended BRH material) and burrowing activity (exposure to bedded BRH material) would be at peak levels. Also, maximum accumulation of lipophilic contaminants would be expected at this time since the period of maximum lipid accumulation occurs during the summer and early fall months (Fig. 10).

Finally, comparisons can be made between the physiological data of the laboratory simulations and the data for the worms collected from the field sampling stations at T+2 (14 days) and T+8 (56 days). These two sampling periods are the only ones in which the temporal scale of the field exposures is matched with the laboratory experiments. Statistically significant differences were observed in the weight specific respiration and ammonia excretion rates of juvenile worms collected from the various stations at T+2 and T+8; however, the pattern of change was not consistent with changes exhibited by the worms from the laboratory simulations. In the laboratory, exposure to suspended BRH material caused significant increases in respiratory rates, while worms collected at the disposal site sampling stations (200E and 400E) exhibited significant decreases in respiration rate.

It is not surprising that there was little similarity between the physiological data from the laboratory simulations and the data obtained from worms collected from the disposal site. Although data was available to design a simulated exposure environment, it is difficult to develop

an exact field environment in the laboratory. Correspondence in exposure environment between the laboratory and the disposal site did exist, however, including the temporal scale (in terms of exposure days), the level of suspended solids expected to be found in the zone immediately above the bottom (Munns et al. 1985), and the oxidation state of the suspended particles (Germano and Rhoads 1984).

One major difference between the laboratory exposures used in this study and the environment around the sampling stations was the composition of the bedded substrate. In the laboratory, all experiments were conducted with REF as the bedded sediment. In the field, only the 1000E and SREF sites were free of appreciable amounts of settled BRH material, while 400E and 200E being within the mound boundary, had BRH sediment layered over the natural Long Island Sound sediment (Germano and Rhoads 1984).

The bedded sediments used by Johns et al. (1985) are probably more similar to the benthic sediment composition of the disposal site sampling stations than were the REF bedded sediment types used in the present study. In the 1985 study, some treatments also included BRH as a bedded sediment. Although the experiment lasted only 10 days, it is interesting to note that the direction and magnitude of the changes found for weight specific respiration and excretion rates of worms exposed in the BRH-bedded treatments were similar to those found for the worms collected from the 200E and 400E stations.

Tissue residue data for worms from the T+8 sampling period and from the 42 day exposure also indicate that differences existed in the overall exposure environment between the laboratory simulations and the field. Although tissue residues for PAHs (sum of mol. wts. 166-302) were similar

for worms from SREF and the 100 percent REF group (840 vs. 611 ug/g dry wt., respectively), worms collected at the 400E station exhibited approximately three times as much PAH as did worms from the 100 percent BRH treatment (16700 vs. 5702 ug/g dry wt.). The tissue levels of PCBs on the other hand, were similar for worms from the 400E site and 100 percent BRH treatment (1160 vs. 1154 ug/g dry wt.).

A primary objective of the FVP was the field verification of the results obtained from laboratory simulations. Given the discrepancies between these two types of tests presented in this discussion, the comparisons between field and laboratory data should be considered qualitative. Worms collected from the BRH disposal site and from the laboratory experiments exhibited changes in physiological function relative to increasing exposures to BRH material. Since an exact match of exposure environments between the laboratory simulations and field environment could not be made, and the fact that the simulated environments included higher concentrations of BRH material than were present at the disposal site, the laboratory findings should be considered an upper bound estimate for the effects of BRH sediment on infaunal polychaetes.

MYSIDOPSIS BAHIA

As with Nephtys incisa, Mysidopsis bahia exhibited a dose-dependent response to suspended BRH material. All physiological parameters examined showed changes within six days following the initial exposure. In addition to the sublethal effects found at the lower BRH level, 100% mortality was noted for mysids maintained in the higher (> 50 percent BRH) levels.

Chronic exposure to BRH material results in lowered growth rates in both juvenile and early adult stages. These reductions in growth can be attributed to reductions in the average amount of food ingested and to an increase in maintenance costs. Reductions in energy consumption levels are apparent by day 5 and persisted thoughout the experiment. The specific compounds causing the reductions in food ingestion rates are unknown, however, previous research with single contaminants suggest that neutral organics, rather than heavy metals, are the causative agents. In a series of studies with decapod larvae, Johns and Pechenik (1980) and Johns and Miller (1982) found that metals (cadmium and copper) did not generally affect feeding rates, whereas exposure to the water-accomodated fraction of No. 2 fuel oil did cause reductions in food ingestion rates. Other research has also indicated that exposure to neutral organics results in depressed feeding rates among marine crustaceans (CITE).

Increased maintenance costs are a generalized metabolic response to contaminant exposure. Stimulation of metabolic activity can be attributed to a variety of factors including functional disruption in metabolic pathways (Ashton and Crafts 1981) or the result of oxidative metabolism by mixed-function oxidases on metal-binding protein systems (Lee 1981). Regardless of the mechanism responsible, higher maintenance costs reduce the amount of assimilated energy available for growth. The net result is that organisms faced with higher maintenance costs will have less energy (both quantity and quality) which can be partitioned into growth processes. Combined with the lowered energy consumption rates found in mysids exposed to BRH material, it is not surprising that the higher maintenance costs resulted in lower growth rates.

The consequences of slowed growth rate include a delay in attaining sexual maturity. Gentile et al. (1985) found that mysids, exposed to the same levels of BRH material employed in this study, exhibited a lower intrinsic rate of population growth. Identified as factors contributing to lowered intrinsic growth included a delay in attaining sexual maturity, an increase in the number of days between sexual maturity and first brood, and the size of the first brood.

Changes in reproductive success reported by Gentile et al. (1985) could be due to a variety of factors including the failure of hormonal systems to initiate egg maturation. Although such factors cannot be discounted, results of the present study indicate that the reproductive alterations identified by Gentile et al. (1985) were due, in part, to the physiological dysfunctions noted during earlier life history stages.

Changes in community and population structure are the product of changes resulting initially at the organism and suborganism level (Sprague 1971; Sastry and Miller 1981). Although isolated effects noted at the organismal level may not translate into population changes due to compensatory mechanisms, it is evident that the sublethal changes seen in this study do result in impacts at the population level of organization. Environmental modification of individual biological energetics of juvenile mysids results in changes in secondary production and the intrinsic rate of population growth. A similar conclusion cannot, at this time, be drawn about bioenergetic dysfunction noted with Nephtys incisa juveniles. N. incisa population from the BRH disposal site sampling stations are currently being analyzed through cohort analysis and this data will

provide an estimate on how well the bioenergetic data predicted eventual population impacts (Gerald Pesch, Personal Communication).

The response of Mysidopsis bahia to BRH material during the entire life cycle, indicate that bioenergetic dysfunction in individuals preceded, and were predictive of, changes at the population level. Changes in growth, development, and reproductive success can be directly linked to alterations in energy balance and flow noted in early juvenile stages. Diversion of assimilated energy to increased maintenance costs significantly reduced both the quantity and quality of energy available for growth and reproductive processes. The integration of several physiological functions into a bioenergetic index proved to be a sensitive indicator of long-term effects occurring at a higher level of biological organization.

Table 20

Tissue residue data for selected organic and inorganic compounds from Nephtys incisa exposed to various suspended particle conditions

Time	Treatment	PAH	PCB	Ethylan	Cu	Cd	Cr
Day 0	Pre-exposure	238	183	0	32	0.53	54
Day 28	100% REF	611	276	0	44	0.68	44
	50% REF/50% BRH	2438	1072	8.6	325	1.04	30
	100% BRH	5703	1155	84.1	636	1.44	51
Day 42	100% REF	517	271	0	38	0.56	15
	50% REF/50% BRH	3306	1117	46.3	370	0.85	4
	100% BRH	4607	1504	98.6	968	1.61	13

SECTION 5

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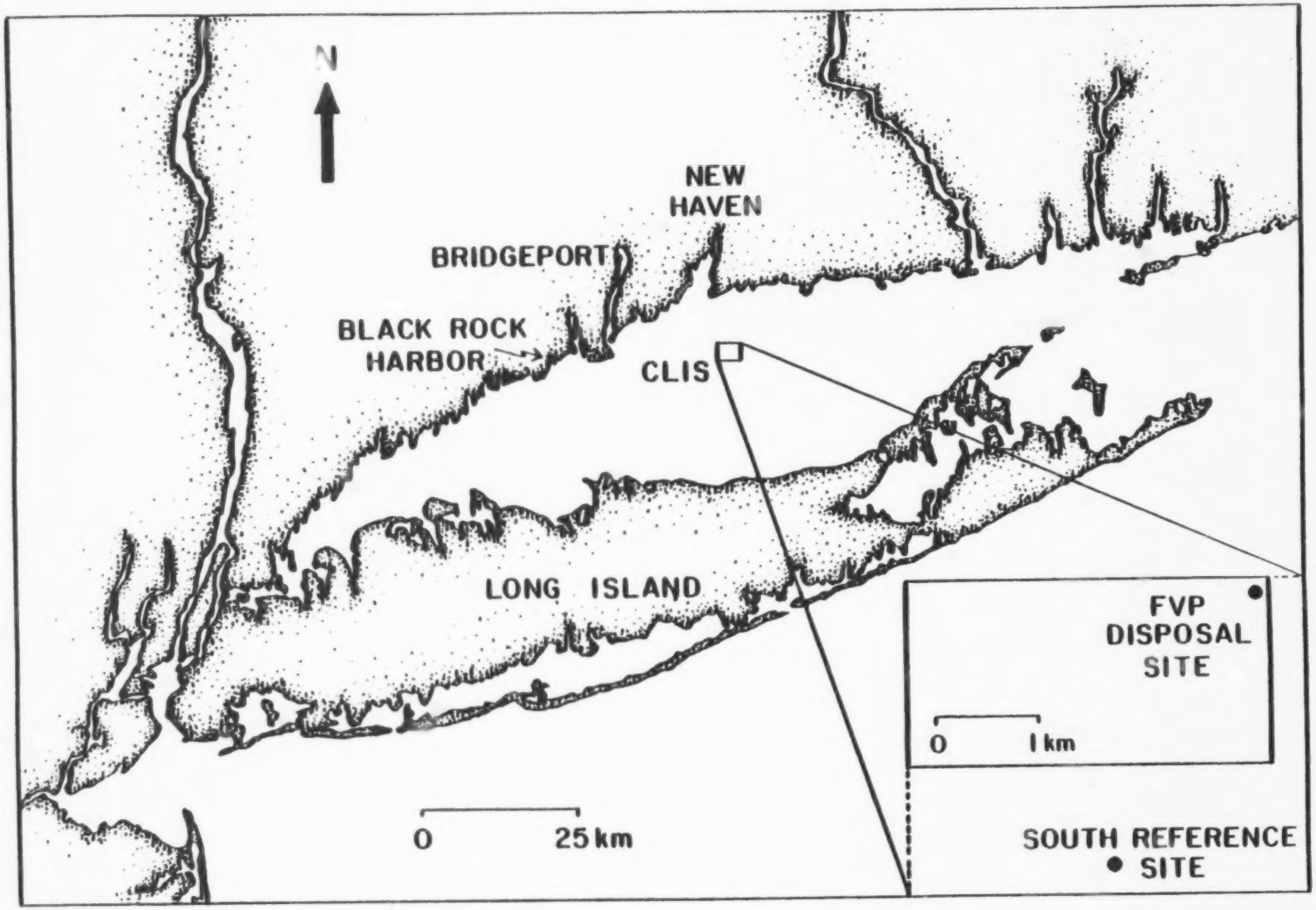
Fig. 1


N
NEW HAVEN
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CLIS
LONG ISLAND
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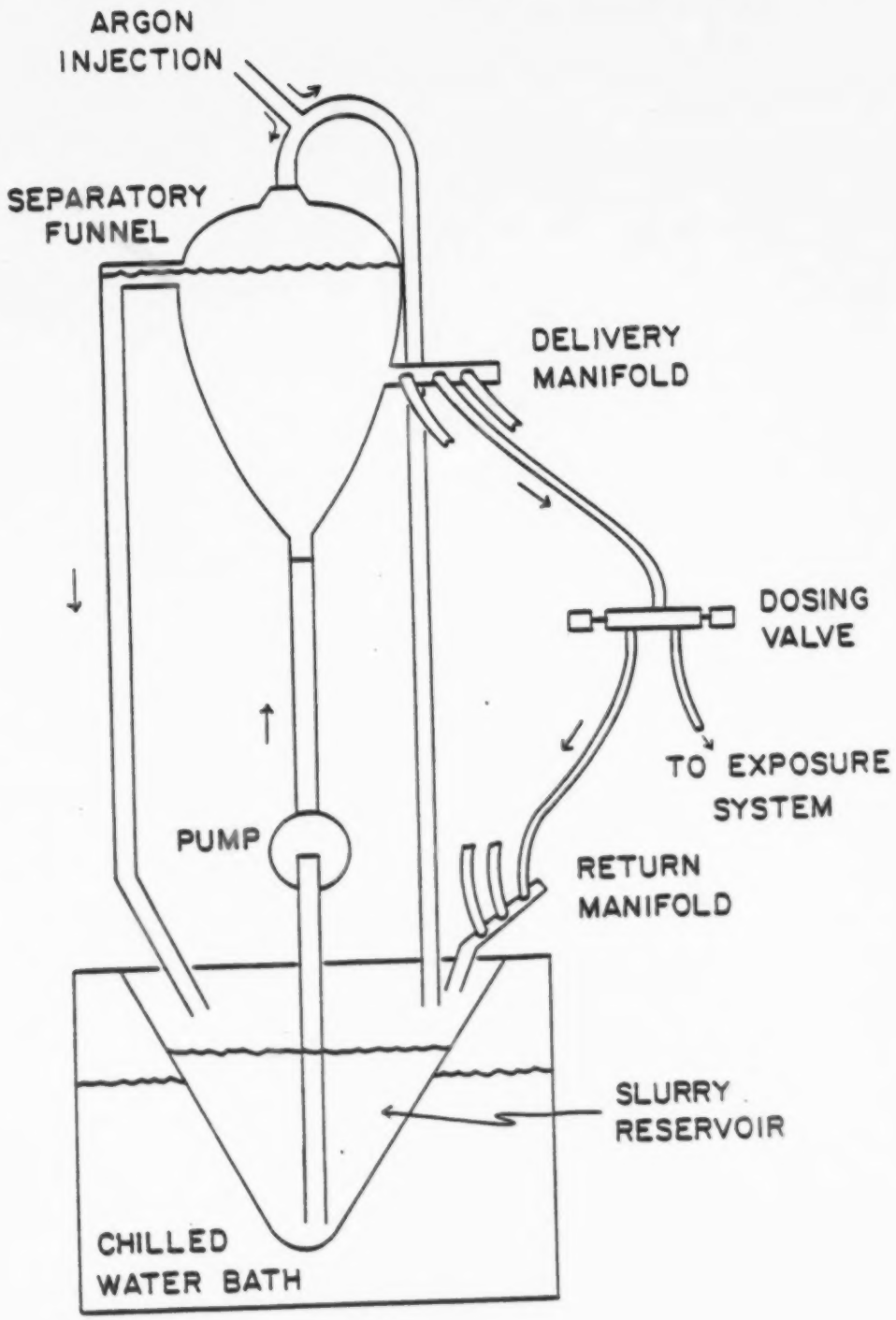
0 25 km
SOUTH REFERENCE SITE

Fig. 2


ARGON
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SEPARATORY
FUNNEL
DELIVERY
MANIFOLD
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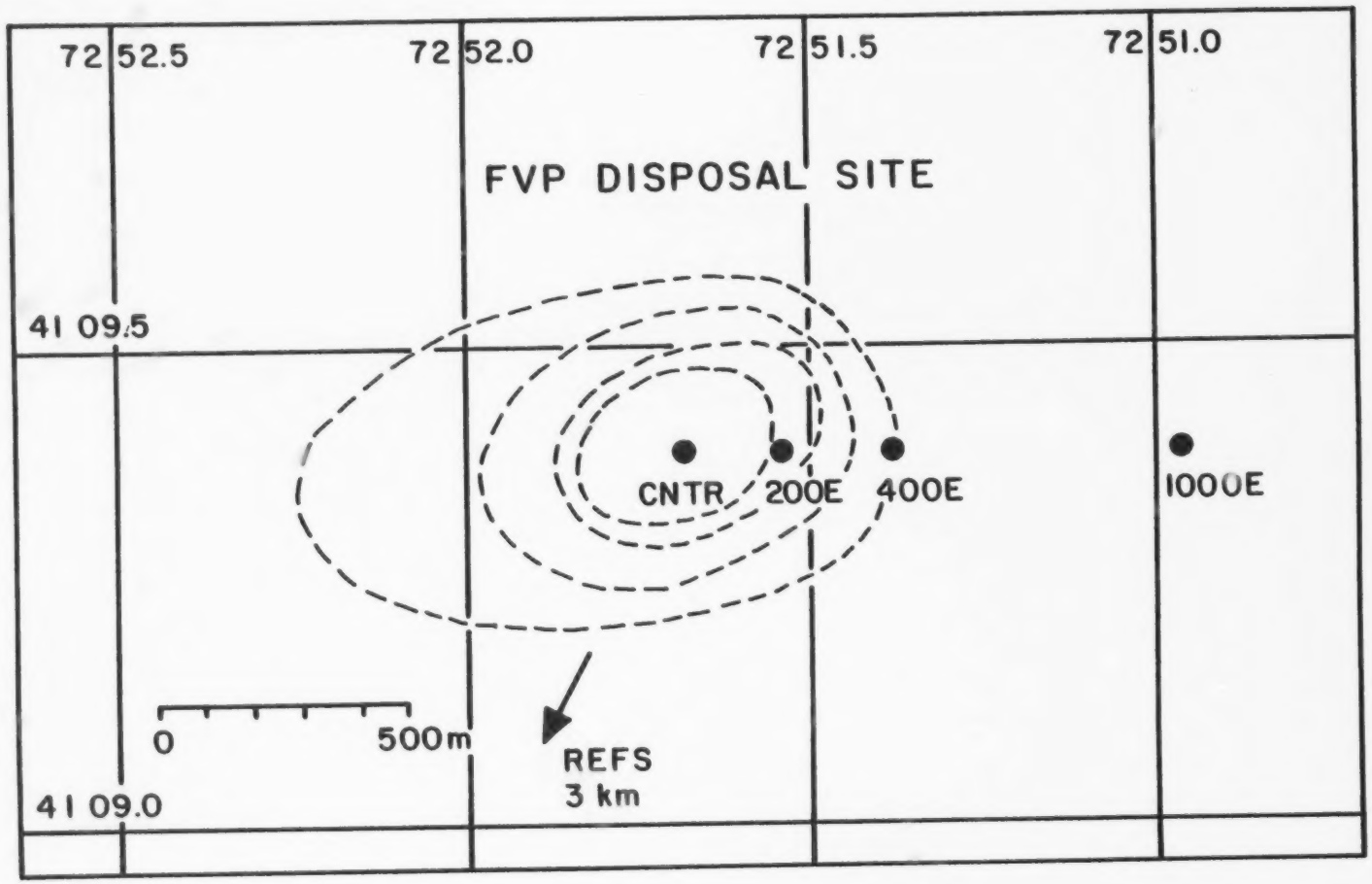
PUMP
RETURN
MANIFOLD
SLURRY
RESERVOIR
CHILLED
WATER BATH

Fig. 5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72 52.0
72 51.5
72 51.0
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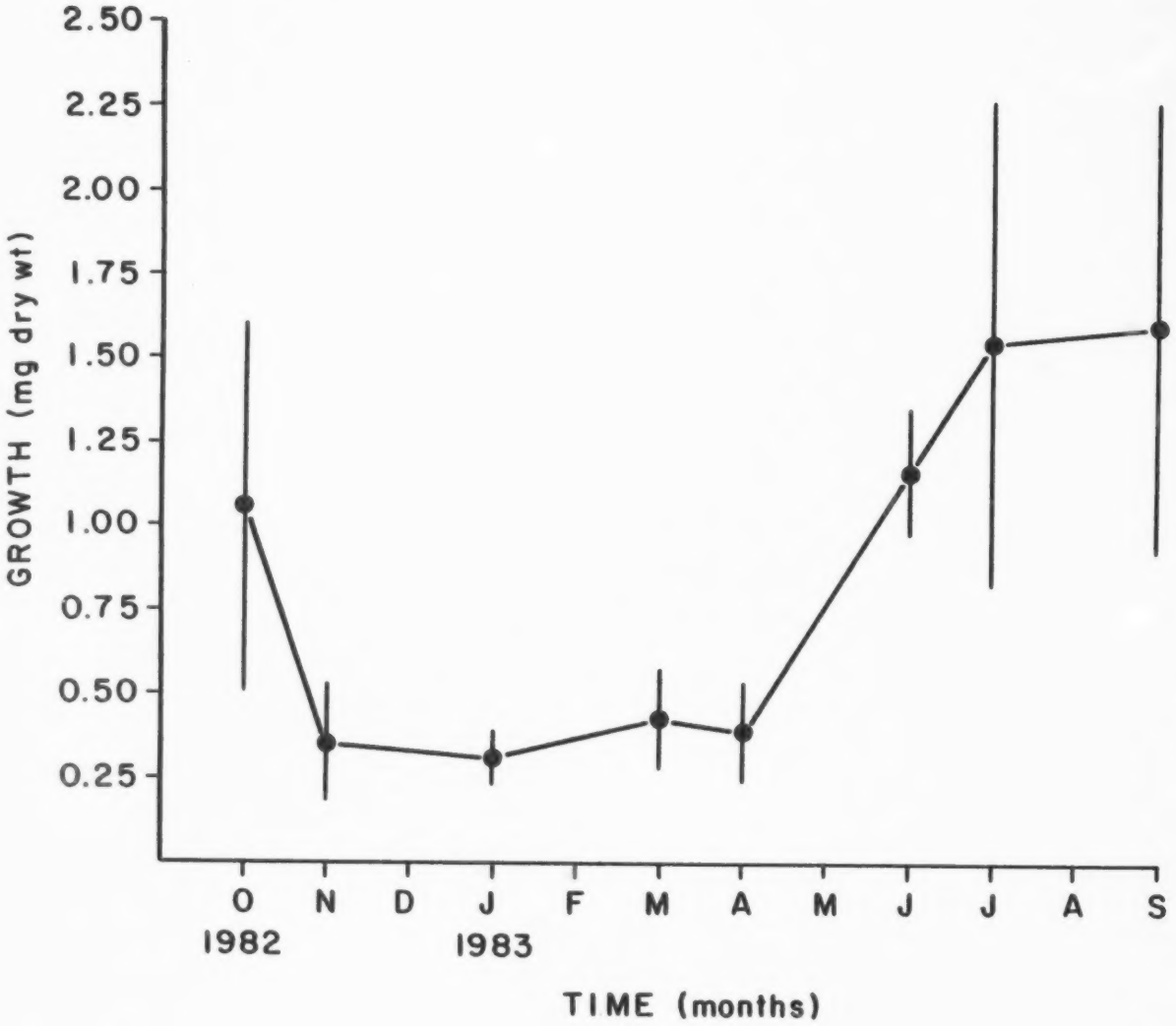
Fig. 7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2.00
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1.50
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1.00
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0.25
GROWTH (mg dry wt)
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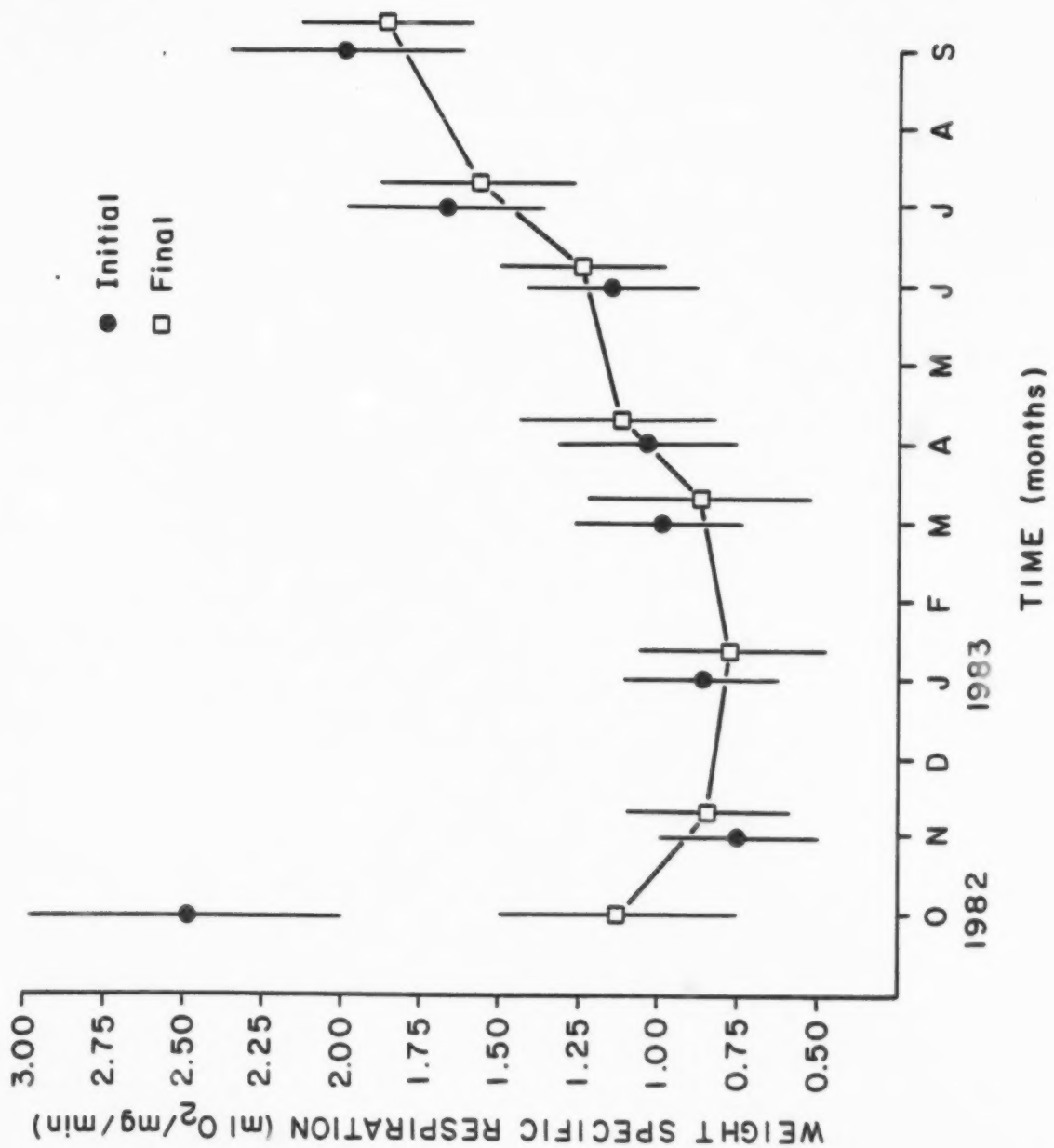
J
F
M
A
M
J
J
A
S
1982
1983
TIME (months)

Fig. 8


WEIGHT SPECIFIC RESPIRATION (ml O_2/mg/min)
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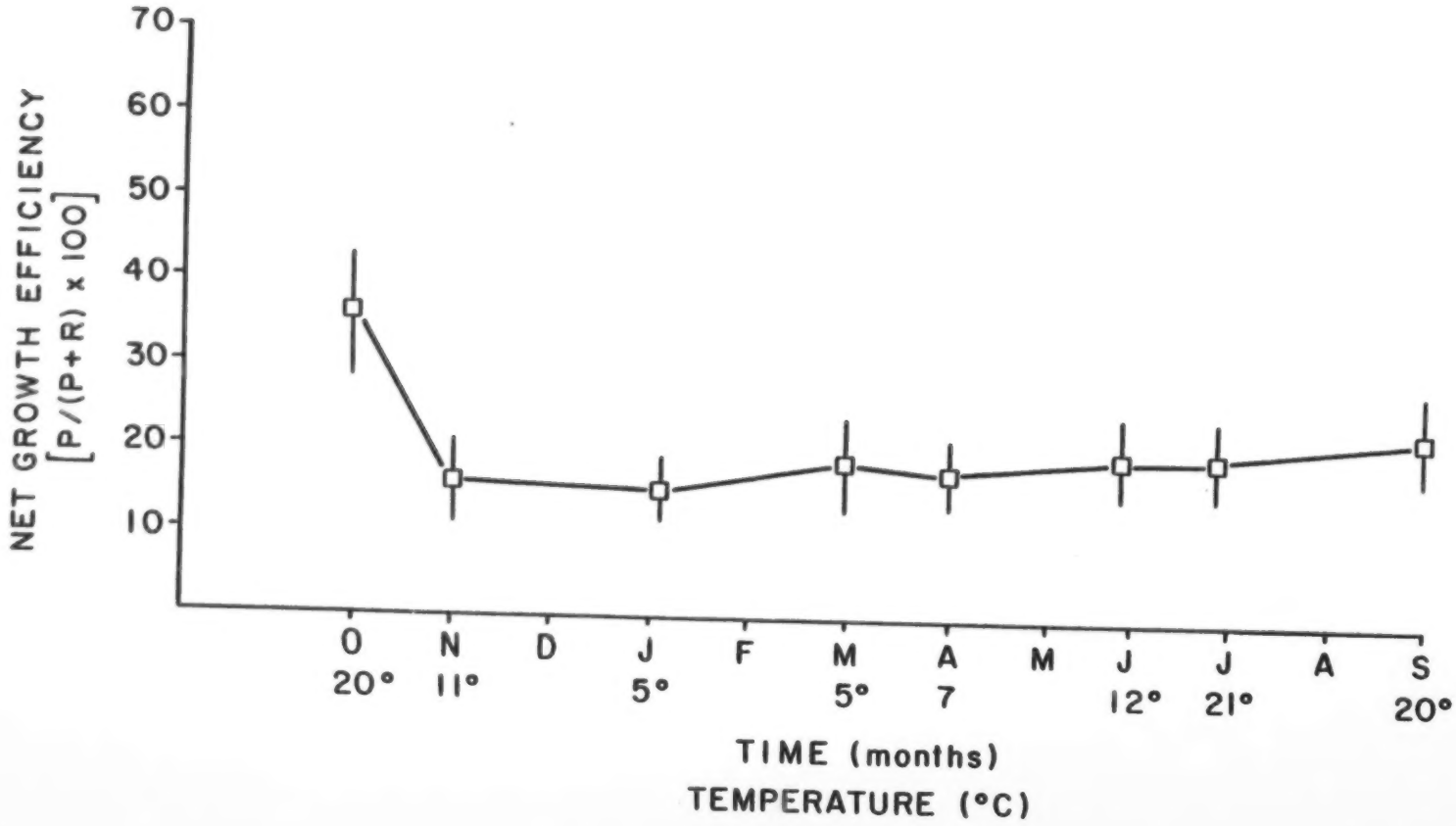
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
Initial
Final
O N D J F M A M J J A S
1982
1983
TIME (months)

Fig. 9


NET GROWTH EFFICIENCY
[P/(P+R) x 100]
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60
50
40
30
20
10
O
20°
N
11°
D
J
5°
F
M
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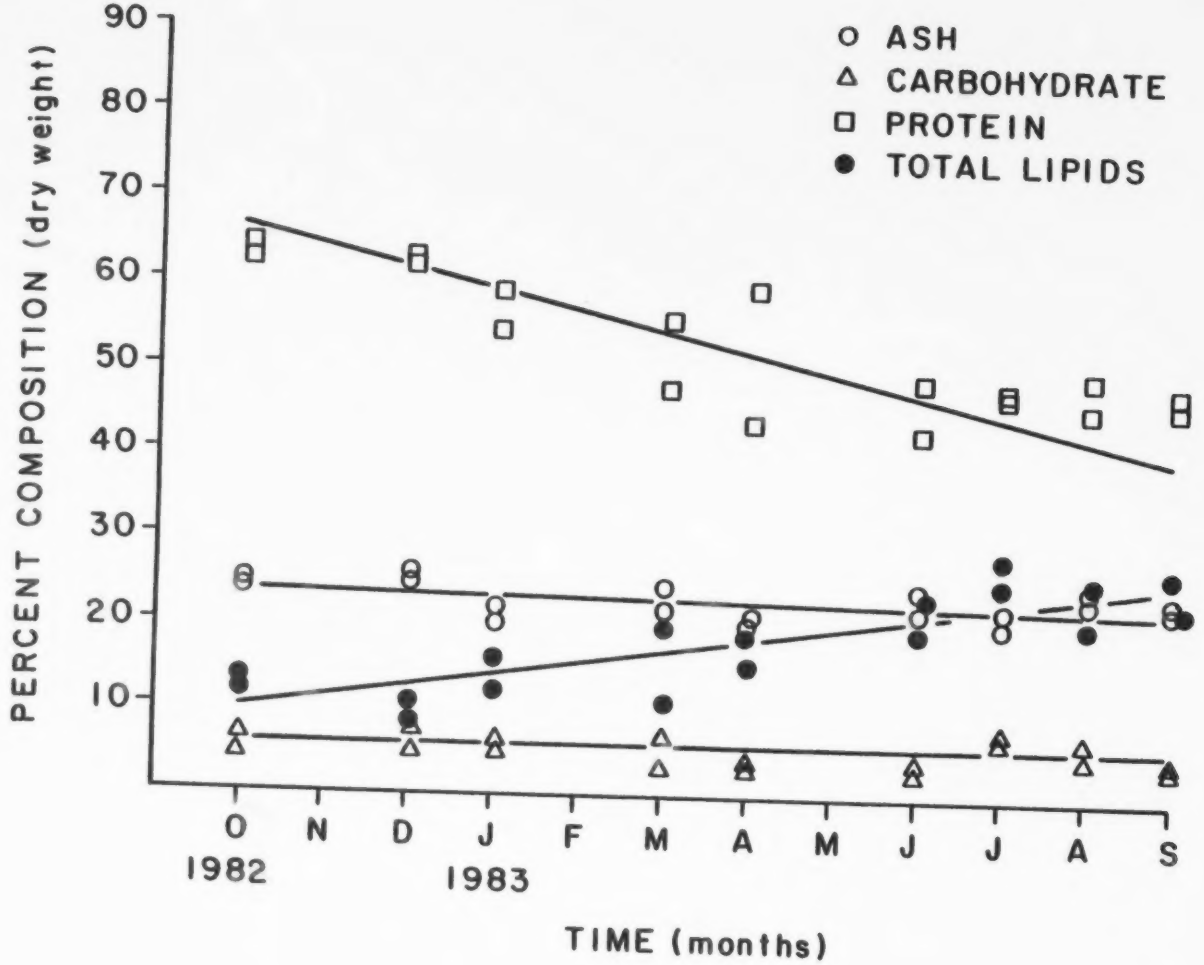
A
7
M
J
12°
J
21°
A
S
20°
TIME (months)
TEMPERATURE (°C)

Fig. 10


ASH
CARBOHYDRATE
PROTEIN
TOTAL LIPIDS
PERCENT COMPOSITION (dry weight)
90
80
70
60
50
40
30
20
10
O
N
D
J
F
M
A
M
J
J
A
S
1982
1983
TIME (months)

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FORM F

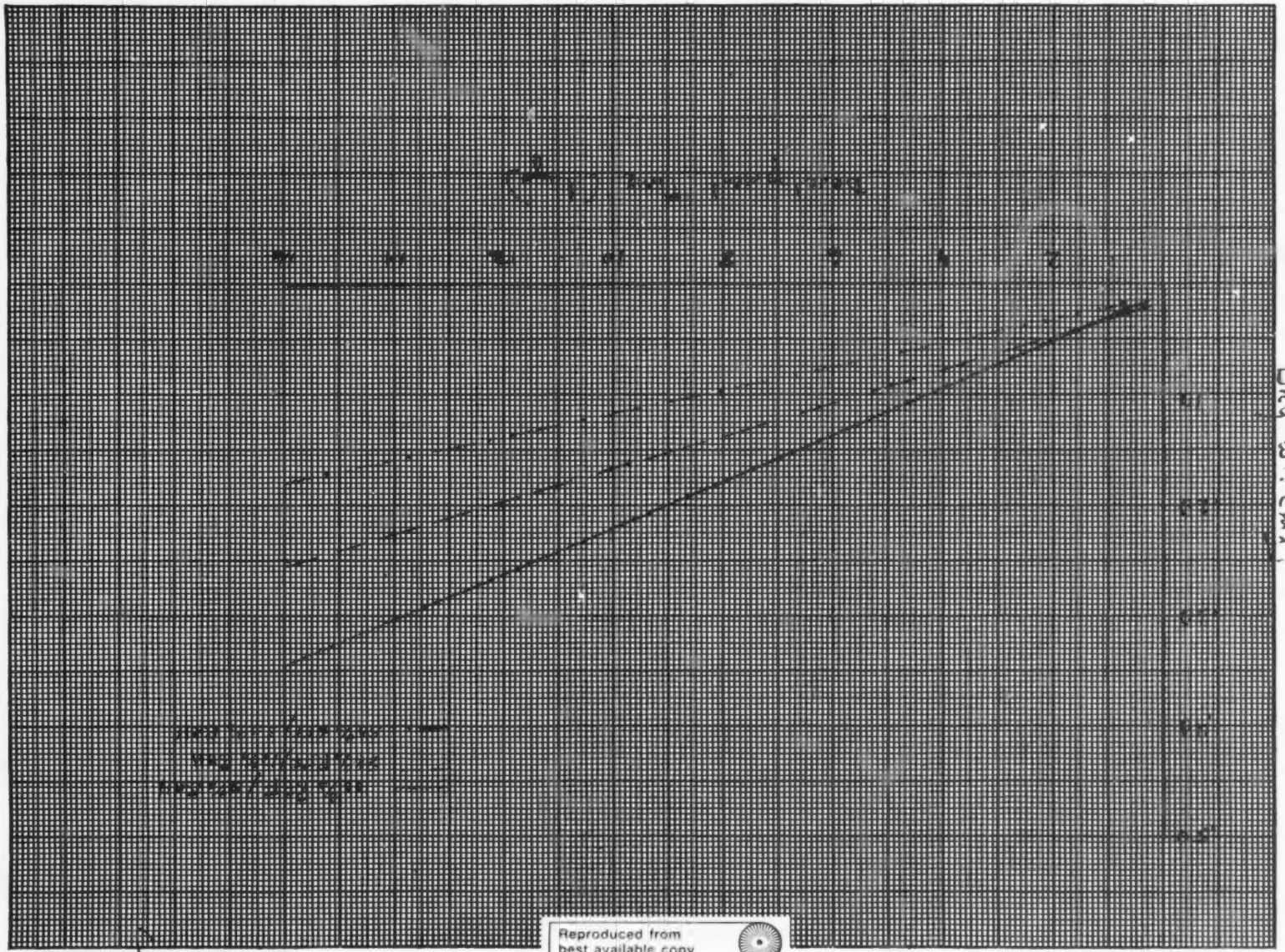

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Fig. 11